International Journal of Dental
Research & Development (IJDRD)
ISSN(P): 2250-2386; ISSN(E): 2321-0117
Vol. 6, Issue 4, Aug 2016, 23-30

TRANS STELLAR
•Journal Publications •Research Consultancy

ESTIMATION OF SERUM IRON LEVELS IN PATIENTS WITH ORAL CANCER

SAVITHA. S. SHETTAR

Reader, Department of Oral Medicine and Radiology, Al-Badar Dental College and Hospital, Gulbarga, Karnataka, India

ABSTRACT

Background and Objectives

The present study was conducted to estimate the serum levels of iron in patients with oral cancer, compare these values among patients with oral cancer and normal subjects and to correlate the values among clinical and histological grades in oral cancer.

Settings

This study was conducted in the Department of Oral Medicine and Radiology, Government Dental College, Bangalore.

Materials and Method

The study consisted of 30 oral cancer patients and 30 normal subjects. Diagnosis of oral cancer was based on clinical and histopathologic findings. The patients were grouped clinically according to TNM staging given by American Joint Committee on Cancer and histopathologically as per the Broder's classification. Serum levels of iron were estimated using semiautoanalyser and data was statistically an alysed. Data analysis was carried out using Statistical Package for Social Science (SPSS, V 10.5) package.

Results

The mean serum levels of iron were decreased in patients with Oral cancer compared to normal subjects. The mean serum levels of iron showed no change through clinical stages of Oral cancer. The mean serum levels of iron showed no change through histological stages of Oral cancer.

Conclusion and Clinical significance

Serum levels of these trace elements may be taken as prognostic markers of the disease progression in Oral cancer patients.

KEYWORDS: Oral Cancer, Trace Elements, Serum Iron

Received: Jul 03, 2016**; Accepted:** Jul 18, 2016**; Published:** Aug 02, 2016**; Paper Id.:** IJDRDAUG20164

Original Article

INTRODUCTION

Oral cancer is one of the 10 most cancers in the world and shows a marked geographic difference in occurrence. On the basis of cancer registry data, it is estimated that annually 75,000 to 80,000 new cancer cases develop in India. Oral cancer ranks number one among men and number three among women in India. In the long incubation period between the initiation of carcinogenic habits and development of invasive oral cancer, well defined oral precancerous lesions such as leukoplakia, submucous fibrosis and erythroplakia occur.[1]

Several studies on diet and cancer links suggest that micronutrients, particularly antioxidants minerals are risk modifiers of cancer.[2] Some dietary-essential mineral elements are constituents of several important antioxidant enzymes. These include selenium, copper, zinc, manganese**,** and iron.[3]Many elements perform functions indispensable to maintenance of growth and reproduction. Inadequate levels of some elements may impair cellular and physiological functions. Trace elements have been critically examined in etiology of various diseases, especially cancer.[4]

Evidence is presented implicating iron deficiency in the suppression of the immune system and its possible role in the initiation or promotion of malignancy.[5] Iron deficiency anemia causes atrophy of the mucous membrane which predisposes to development of carcinoma in these tissues.[6]

As micronutrients deficiencies are common in India and have been related to oral and upper aero digestive tract cancers, it is considered to be necessary as well to study the impact of nutrients on oral cancer. It is reasonable to assume that the serum levels of these biochemicals might have modifying effects in their etiology, treatment and prognosis.

The current study reported is an attempt to suggest a positive role of micronutrients in prevention of oral cancer. The biochemical assessment of patients with oral malignant lesion may help in earlier diagnosis and /or prognosis of the lesions, hence the present study has been undertaken.

MATERIALS AND METHODOLOGY

This study was conducted in the Department of Oral Medicine and Radiology, Government Dental College, Bangalore. 30 patients with Oral cancer and 30 age and sex matched healthy controls formed the study group. Patient selection was based on following inclusion and exclusion criteria.

Inclusion Criteria

- Patients with a definitive diagnosis of Oral cancer both clinically and histopathologically were included in the study.

Exclusion Criteria

- Patients who have received treatment for Oral cancer previously were excluded from the study.
- Patients with history of diabetes, hypertension, anaemia, jaundice, liver or kidney disorders or other systemic diseases and carcinoma elsewhere in the body were excluded from the study.

A detailed case history of the patient was taken and a thorough clinical examination was done and recorded on a standard proforma.

A formal ethical clearance to conduct this study was obtained by the Ethical Committee of the college. A formal informed written consent was obtained from all pateints.

30 patients of Oral cancer diagnosed based on the history and clinical features with confirmation of diagnosis through histopathological examination were included in the study. The patients were grouped clinically according to TNM staging given by American Joint Committee on Cancer[7] and histopathologically as per the Broder's classification.[8]

T (Size of Primary Tumor)

T1s: Carcinoma in situ

T1: Tumor < 2cm

T2: Tumor < 2cm and < 4 cm

T3: Tumor > 4cm

T4: Tumor > 4cm with invasion of adjacent structures (i.e. through cortical bone, deep into extrinsic muscles of tongue, maxillary sinus and skin).

N (Cervical Lymph Node Metastases)

N0: No node involvement detected

N1: Single ipsilateral node < 3cm

N2a: Single ipsilateral node < 6cm

N2b: Multiple ipsilateral nodes > 3cm and < 6cm

N2c: Bilateral or contralateral lymphnodes < 6cm

N3a: Ipsilateral node > 6cm

N3b: Bilateral nodes > 6cm

M (Distant Metastasis)

M0: No known metastasis

M1: Metastasis present

Staging

Stage 1: T1N0M0

Stage 2: T2N0M0

Stage 3: T3N0M0; T1,T2 or T3N1M0

Stage 4: T4 any N M0; any T N2 or N3 M0; any T or N, with M1.

The Patients with Oral Cancer Were Grouped Histopathologically According to Broder's Grading as Follows:

Grade I: Well - differentiated (less than 25% anaplastic cells).

Grade II: Moderately - differentiated (25% - 50% anaplastic cells).

Grade III: Moderately - differentiated (50 - 75% anaplastic cells).

Grade IV: Poorly – differentiated or anaplastic (more than 75% anaplastic cells).

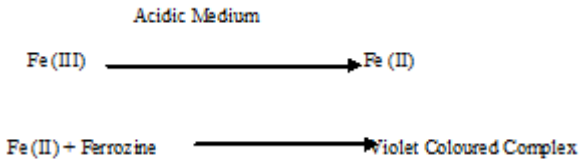
5ml of venous blood was collected using aseptic measures from median cubital vein and sent to laboratories in sterile vials for estimation of serum Iron and Total protein levels. The blood was allowed to clot at room temperature for

1-2 hrs and then serum was separated by centrifuging at 3000 rpm for 10 minutes. Serum Iron levels were estimated using semi autoanlyser as follows:

Serum Iron Estimation

Methodology: Ferrozine method.

Principle: Iron, bound to Transferrin, is released in an acidic medium and the Ferric ions are reduced to Ferrous ions. The Fe (II) ions react with Ferrozine to form a violet colored complex. Intensity of the complex formed is directly proportional to the amount of Iron present in the sample.



Contents

L1: Iron buffer reagent 35ml

L2: Iron buffer reagent 2ml

S: Iron standard (100µg/dl) 2 ml

Sample: Serum

Procedure

Wavelength / Filter: 570nm (Hg 578nm) /yellow

Temperature: Room Temperature.

Light Path: 1cm.

Table 1: Iron assay:Pipette into clean dry test tubes labeled as Blank (B), standard (S), Sample Blank (SB), and Test (T)

Addition Sequence	Blank (B) (ml)	Standard (S) (ml)	Sample Blank (SB) (ml)	Test (T) (ml)
Iron buffer reagent (L1)	1.0	1.0	1.05	1.0
Distilled water	0.2			
Iron standard (S)		0.2		
Sample			0.2	0.2
Iron Color Reagent (L2)	0.05	0.05		0.05

Calculation

$$\text{Iron in } \mu g/dl = \frac{Abs.T-(Abs.SB+ Abs.B)}{Ab.S - Abs.B} \times 100$$

Expected Values

Males: 60-160 µg/dl, Females: 35- 145 µg/dl

Data analysis was carried out using Statistical Package for Social Science (SPSS, V 10.5) package. Student t test was applied. One way Analyses of Variance were used to test the difference between groups and to find out which of the two groups means is significantly different post hoc test of Tukey test was used. In the above test the "p" value of less than 0.05 was accepted as indicating statistical significance.

RESULTS

Among 30 Oral cancer patients there were 20 males (66.7 %) and 10 females (33.3 %) patients. In patients with Oral cancer the mean age was found to be 55.70±12.65 years (mean ± SD) with 30% in the age group of 50-59 years, 23.3% in the age group of 40-49 years, 20% in the age group of 60-69 years, 20 % in the age group of 70-79 years, 3.3% of patients in the age group of 20 -29 years and 3.3% of patients in the age group of 30 -39 years. **Table 1**

The clinical staging done for Oral cancer as per the TNM staging given by American joint committee on cancer showed that 2 (6.7 %) belonged to stage I, 3 (10 %) belonged to stage II, 12 (40 %) belonged to stage III and 13 (43.3 %) belonged to stage IV. **Table 2**

The histological grading of Oral cancer patients as per Broder's classification showed that 13 (43.3%) were Grade I, 10 (33.3%) were of Grade II and III, 7 (23.3%) were of Grade IV. **Table 3**

The mean serum iron was 113.03 ± 23.35 µg/dl (mean ± SD) in Control group and 99.93 ± 18.42 µg/dl (mean ± SD) in patients with Oral cancer. The mean serum iron level in Oral cancer patients was significantly decreased compared to normal subjects. **Table 4** There was no statistically significant difference in the mean serum iron level in the clinically and histologically divided groups in Oral cancer patients. **Table 5&6**

DISSCUSIONS

In present study the mean serum iron levels in patients with Oral cancer were decreased compared with that of normal subjects. These findings were similar to the findings in the studies done by **Sunali Khanna and Frenny Karjodkarin 2005**[9]and **in 2006**[10]who observed that the levels of serum iron were decreased in precancer and cancer groups compared to that of normal subjects. They stated that there is association between the serum iron and oral carcinogenesis and more detailed studies on large data base should be instituted to elucidate the exact role of iron.

Study done by **Apeksha et al in 2010** also showed a decrease in serum iron levels in patient with osf, leukoplakia and oral cancer compared to control group and they stated that all patient included were of same socioeconomic status lower serum iron levels appear to be effect of disease process rather than its cause. Lack of consumption of normal diet results in anemia which is further perpetuated by progression of disease.[11]

The literature regarding the association between iron and oral cancer is controversial. Study done by **Amith Kumar et al in 2014**[12]showed findings of increased oral cancer serum iron level does not match with the several of previous research findings that stated deficiency of iron resulted in oral carcinoma. One aspect of diet that has not been widely studied is iron metabolism. Iron is an essential nutrient, and iron deficiency is a very common form of malnutrition worldwide. A

High level of available tissue iron may increase the risk of cancer through its contribution to the production of free oxygen radicals. Iron deficiency or iron excess leads to oxidative DNA damage. [12]

Other studies by **Paul RR et al in 1996[13], Jyothi T et al in 2011[14],Karthik H et al in 2012[15] and Yadav A et al in 2015[16]** showed that serum iron levels were also decreased in OSF patients compared to control group. They have suggested that decreased iron levels in oral submucous fibrosis patients might be due to utilization of iron in collagen synthesis. Furthermore, lack of iron in the tissues results in decreased vascularity which facilitates percolation of arecoline. In vitro studies on human fibroblasts observed that arecoline causes increased fibroblastic proliferation and collagen formation which is a hallmark of OSMF. Serum levels of these biochemicals may be used as diagnostic and prognostic markers in OSF patients. And this biochemical assessment can be of value for proactive intervention of high risk groups.

Our study showed a decrease in serum iron levels in patients with oral cancer patients compared to normal subjects. Iron deficiency has been reported as causing epithelial atrophy, koilonchyia, glossitis and dysphagia. Atrophy of the mucous membrane predisposes to development of carcinoma in these tissues.[6]Iron deficiency causes depression of cell mediated immunity which may predispose to malignancy. In addition to the structural and kinetic changes of oral epithelium in iron deficiency, iron deficiency may have an influence on the oral flora. It is well recognized that there co-exists a close association between the iron status of an animal and the microbiological flora and candida albicans, a commensual of the normal oral flora, can augment the carcinogenic potential of a specific carcinogen.[17]

The present study showed no significant change in serum iron levels among clinical and histological grades in oral cancer patients.

CONCLUSIONS

Our study showed change in serum levels of iron in patients with oral cancer compared to that of normal subjects. On the basis of this study it can be suggested that serum levels of these biochemicals may be used as diagnostic and prognostic markers in oral cancer patients. And this biochemical assessment can be of value for proactive intervention of high risk groups. But to validate the above results further studies on large sample size are required.

REFERENCES

1. *Sankarnarayanan R. Oral cancer in India: An Epidemiologic and Clinical Review. Oral Surg Oral Med Oral Pathol 1990; 69: 325-330.*
2. *Krishnaswamy K, Prasad MPR, Krishna TP, Annapurna VV, Amrendra R. A Case Study of Nutrient Intervention of Oral Precancerous Lesions in India. Oral oncol, Eur J Cancer 1995; 31B (1):41-48.*
3. *Enwonwu CO, Meeks VI. Bionutrition and Oral Cancer in Humans. Crit Rev Oral Biol Med 1995;6(1):5-17.*
4. *Vyas K, Abha G, Aeron AK. Serum copper, zinc, magnesium and calcium levels in various human diseases. Indian J Med Res1982 August; 76:301-304.*
5. *Freddie AH. Micronutrient Requirements of Cancer Patients. Cancer 1985;55:295-300.*
6. *Rajendran R, Shivapathasundharam B. Shafer's Textbook of Oral Pathology. 5th ed. Elsevier publishers. 2006.*
7. *Martin SG, Michael G. Burket's Oral Medicine Diagnosis & Treatment. 10th ed.Harcourt (India) Private Limited, 2003.*
8. *Harsh M. Textbook of Pathology. 3rd edition. Jaypee Medical Publishers (P) LTD.1998.*

9. *Sunali K, Frenny K. Immunological and Biochemical Markers in Oral Pre-cancer and Cancer: A Study. JIAOMR 2005; 17(04): 161-164.*

10. *Paul RR, Chatterjee J, Das AK, Dutt SK, Roy D. Zinc and Iron as Bioindicators of Precancerous Nature of Oral Submucous Fibrosis. Biol Trace Elem Res.1996 Sep;54(3):213-30.*

11. *S.Khanna et al Circulating Immuno Complexes and Trace Elements(Copper Iron and Selenium) as a Marker in Oral Precancer and Cancer: A Randomized, Controlled Clincal Study. Head & Face Medicine 2006, 2:33*

12. *Jyothi Tadakamadla, Santhosh Kumar, Mamatha GP. Evaluation of serum copper and iron levels among oral submucous fibrosis patients. Med Oral Patol Oral Cir Bucal. 2011 Nov 1;16 (7):e870-3.*

13. *Yadav A, Kumar L, Misra N, Deepak U, Shivakumar GC. Estimation of Serum Zinc, Copper and Iron in the Patients Of Oral Submucous Fibrosis. Natl.J Maxillofac Surg.2015 Jul-Dec;6(2):190-3.*

14. *Apeksha R. et al estimation and comparative evaluation of serum iron, copper, zinc and copper/zinc ratio in Oral leukoplakia, Submucous fibrosis and Squamous cell carcinoma. Journal of Indian Association of Medicine and Radiology.2010;22(2):73-76.*

15. *Amith Kumar et.Al Estimation of Serum Micronutrient Levels and The Possible Risk of Oral Cancer and Premalignancy. International Journal of Innovative Research in Science, Engineering and Technology.2014;3(1):8360-8363.*

16. *Karthik H, Nair P, Gharote HP, Agrawal K, Ramamurthy B, Kalanpur R.Role of Hemoglobin and Serum Iron in Oral Submucous Fibrosis: A Clinical Study. The Scientific World Journal. 2012, Article ID 254013, 5 pages.*

17. *Prime SS, Macdonald, Rennie JS. The Effect of Iron Deficiency on Experience Oral Carcinogenesis in the Rat. In.J.Cancer 1983; 47:413-418. (17)*

APPENDICES

TABLES

Table 1: Age Distribution of the Study Groups

Age	10-19 yrs	20-29 yrs	30-39 yrs	40-49 yrs	50-59 yrs	60-69 yrs	70-79 yrs
Oral cancer		1 (3.3%)	1 (3.3%)	7 (23.3%)	9 (30%)	6 (20%)	6 (20%)

Table 2: Clinical Grading of Oral cancer Group

Stage I	Stage II	Stage III	Stage IV
2 (6.7 %)	3 (10 %)	12 (40.0 %)	13 (43.3%)

Table 3: Histological Grading of Oral cancer Group

Grade I	Grade II &III	Grade IV
13 (43.3%)	10 (33.3%)	7 (23.3 %)

Table 4: Mean Serum Iron Levels among Study Groups

	N	Mean	Std Deviation	t	p
Control	30	113.03	23.35		
Oral cancer	30	99.93	18.42	2.4	**0.01**

Table 5: Comparison of Mean Serum Iron Levels among the Clinical Stages in Oral Cancer

	N	Mean	Std Deviation	Minimum	Maximum	F	P Value
Stage I	2	96.00	22.62	80	112		
Stage II	3	100.67	17.92	80	112		
Stage III	12	92.58	18.42	76	124	1.389	.268
Stage IV	13	107.15	17.29	78	130		

Table 6: Comparison of Mean Serum Iron Levels among Histological Grades in Oral Cancer

	N	Mean	Std Deviation	Minimum	Maximum	F	P Value
Grade I	13	95.31	17.19	76	120		
Grade II & III	10	106.40	22.29	80	130	1.033	.370
Grade IV	7	99.29	13.66	83	112		